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An Essential *Saccharomyces cerevisiae* Gene Homologous to *SNF2* Encodes a Helicase-Related Protein in a New Family

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The *Saccharomyces cerevisiae SNF2* gene affects the expression of many diversely regulated genes and has been implicated in transcriptional activation. We report here the cloning and characterization of *STH1*, a gene that is homologous to *SNF2*. *STH1* is essential for mitotic growth and is functionally distinct from *SNF2*. A bifunctional STH1–β-galactosidase protein is located in the nucleus. The predicted 155,914-Da STH1 protein is 72% identical to SNF2 over 661 amino acids and 46% identical over another stretch of 66 amino acids. Both STH1 and SNF2 contain a putative nucleoside triphosphate-binding site and sequences resembling the consensus helicase motifs. The large region of homology shared by STH1 and SNF2 is conserved among other eukaryotic proteins, and STH1 and SNF2 appear to define a novel family of proteins related to helicases.

The *SNF2* gene appears to play an important and general role in transcriptional activation in *Saccharomyces cerevisiae*. *SNF2* affects the expression of many diversely regulated genes, including glucose-repressible genes, the acid phosphatase gene, certain cell type-specific genes, and Ty elements (3, 27, 40, 45, 50). Moreover, *SNF2* is the same as *SWI2* (48), which is required for *HO* gene expression (71); the HO endonuclease initiates mating type switching in homothallic yeast strains (29). *SNF2* is also identical to *TYE3* (9) and to *GAM1* (85). Although *SNF2* is not essential for viability, mutants are unhealthy and homozygous diploids fail to sporulate.

Genetic evidence indicates that *SNF2* is functionally related to the *SNF5* and *SNF6* genes (18, 50, 52) and likewise that *SWI2* is related to *SWI1* and *SWI3* (71, 72). We have shown previously that LexA-SNF2 or LexA-SNF5 fusion proteins, tethered to DNA via a *lexA* operator, activate transcription of a target gene (39, 40). The data suggest that the SNF2, SNF5, and SNF6 proteins function interdependently in transcriptional activation, perhaps forming a heteromeric protein complex.

In preliminary experiments using anti-SNF2 antibody, we detected several cross-reacting proteins in the size range predicted for SNF2 (38). Previously, we noted that an open reading frame (ORF) adjacent to *LYS2* is homologous to SNF2 (40). This ORF corresponds to *RAD16*, a gene involved in DNA excision repair (63), and it is predicted to encode a protein significantly smaller than SNF2. We therefore began a search for other *S. cerevisiae* genes homologous to *SNF2*.

We have cloned a gene, designated *STH1*, that is highly similar to *SNF2* and that is essential for viability. Here we report the sequence of the *STH1* gene and the nuclear localization of its gene product. We also report genetic evidence that *SNF2* and *STH1* have distinct functions. The predicted STH1 protein contains a nucleoside triphosphate (NTP)-binding motif and other motifs that are conserved in helicases. SNF2 and STH1 also share homology to other eukaryotic proteins and appear to define a new family of proteins that are related to helicases.

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MATERIALS AND METHODS

Yeast strains and genetic methods. Strains of *S. cerevisiae* are listed in Table 1. Standard genetic methods were followed (66). Media used were yeast extract-peptone containing glucose (YPD) or synthetic complete (SC) medium lacking the appropriate supplements to maintain selection for plasmids (66).

Southern blot and genomic library hybridization. Genomic DNA was prepared (32) and Southern blot analysis was carried out (42) as described previously. Bacteria harboring a yeast genomic DNA library cloned in YEp24 (7) were screened by colony hybridization (42). Following hybridization, filters were washed under low-stringency conditions in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.05% sodium dodecyl sulfate (SDS) for 15 min at 50°C. Labeled probe was prepared by nick translation of the 1.1-kb *Eco*RI fragment from pLN138-4 (3), which contains *SNF2* codons 835 through 1206.

DNA sequencing. Restriction fragments from pBL50 were subcloned into M13mp18 and M13mp19 (53). The sequences of both strands for the region −700 to +4427 were determined by the dideoxy chain termination method (61) with Sequenase (United States Biochemical) and the 17-mer universal primer (Amersham) and six synthetic 18-mer oligonucleotides. The DNA sequence is available from GenBank.

RNA analysis. Poly(A)-containing RNAs were prepared (62) from cultures grown in YPD, separated in a 1.5% agarose gel containing formaldehyde, and transferred to nitrocellulose paper (42). Filters were hybridized and washed as described previously (42).

Disruption of the chromosomal *STH1* locus. pBL51 contains the 4.4-kb *Eco*RI-*Bam*HI fragment of pBL50 cloned in pUC19 (84). pBL52 was constructed from pBL51 by digesting it with *Kpn*I, blunting the ends with T4 polymerase, and ligating it to the 1.1-kb *URA3 Sma*I fragment of pAC100 (2). pBL53 was constructed similarly by using the 1.6-kb *HIS3 Bam*HI fragment of YEp6 (73). pBL54 was created by replacing the *Eco*RV-*Nco*I fragment of pBL51 (codons 92 through 1289) with *HIS3*. Diploid strains MCY1751 and MCY2448 were transformed to uracil independence with the 5.5-kb *Eco*RI-*Sal*I fragment of pBL52 (*Sal*I site in pUC19 polylinker). Diploid strains MCY2448 and MCY2447 were

TABLE 1. List of *S. cerevisiae* strains

Strain	Genotype[a]
MCY815	*MATα ssn20-6 his4-539 ura3-52 SUC2*
MCY829	*MATα his3-Δ200 ura3-52 lys2-801 SUC2*
MCRY900	*MATa sth1-2::HIS3 ura3-52 his3-Δ200 ade2-101 SUC2* [pSTH1(1291)-lacZ]
MCRY920	*MATa/MATα his3-Δ200/his3-Δ200 ura3-52/ura3-52 ade2-100/+ +/lys2-801 sth1-1::URA3/+ SUC2/SUC2*
MCY1093	*MATa his4-539 lys2-801 ura3-52 SUC2*
MCY1094	*MATα ade2-101 ura3-52 SUC2*
MCY1250	*MATα lys2-801 ura3-52 snf2-Δ1::HIS3 his3-Δ200 SUC2*
MCY1751	MCY1093 × MCY1094
MCY1996	*MATa snf2-Δ2::URA3 ura3-52 his4-539 lys2-801 ade2-101 SUC2*
MCY1997	*MATa ura3-52 lys2-801 ade2-101 snf2-Δ2:: URA3 SUC2*
MCY1998	*MATα ura3-52 lys2-801 snf2-Δ2::URA3 SUC2*
MCY2447	*MATa/MATα ade2-1/ade2-1 his3-11,15/his3-11,15 leu2-3,112/leu2-3,112 trp1-1/trp1-1 ura3-1/ura3-1 can1-100/can1-100*
MCY2448	*MATa/MATα his3-Δ200/his3-Δ200 ura3-52/ura3-52 ade2-101/+ +/lys2-801 SUC2/SUC2*
MCY2471	*MATa/MATα his3-Δ200/his3-Δ200 ura3-52/ura3-52 ade2-101/+ +/lys2-801 sth1-2::HIS3/+ SUC2/SUC2*
MCY2484	*MATa ssn20-1 ura3-52 lys2-801 SUC2*

[a] All strains have the S288C genetic background except for MCY2447, which is derived from W303.

transformed to histidine independence with the 6-kb *Eco*RI-*Sal*I fragment of pBL53 and the 2.6-kb *Eco*RI-*Bam*HI fragment of pBL54, respectively. DNA from transformants was analyzed by Southern blot hybridization to confirm that the mutation had been introduced at the *STH1* locus on one chromosome homolog.

Construction of LexA fusion plasmids. To construct pLexA-STH1(1-1330), we used two synthetic oligonucleotide primers to direct the synthesis of the N-terminal 91 codons of *STH1* by the polymerase chain reaction (60) with pBL51 as the DNA template. The reaction was initiated by the addition of Amplitaq DNA Polymerase (Perkin-Elmer Cetus) with 30 temperature cycles of 94°C for 1 min, 45°C for 1 min, and 73°C for 2 min. The primers were a 27-mer complementary to nucleotides 1 through 19, 5′ GGGAAT TCATGCTTCAGGAACAATCTG 3′, designed to create an *Eco*RI site (underlined) immediately 5′ to the initiating ATG codon, and an 18-mer complementary to nucleotides 336 through 319, 5′ CTCATCGTACTGAAATCC 3′, 50 bp 3′ to the *Eco*RV site. The amplified products were digested with *Eco*RI plus *Eco*RV, electroeluted from an 8% acrylamide gel, and ligated to the *Eco*RV-*Bam*HI fragment of pBL50 plus the *Bam*HI-*Eco*RI fragment of pSH2-1 (25). Codons 1 through 87 of *lexA* and four codons derived from the pSH2-1 polylinker and the polymerase chain reaction primer are fused in frame to codon 1 of *STH1*. A translational stop codon provided by pSH2-1 lies 6 residues 3′ to codon 1330 of *STH1*.

To show that pLexA-STH1(1-1330) provides STH1 function, we transformed diploid strain MCRY920 (*sth1-1::URA3/STH1*) and sporulated His^+ diploids with selection for the plasmid. The six tetrads analyzed yielded viable His^+ Ura^+ spores, indicating that pLexA-STH1(1-1330) complements the lethality of the *sth1* mutation.

pLexA-STH1(57-1352) was constructed by first subcloning the *Cla*I fragment of pBL50 into the *Kpn*I site of pUC19, creating pXY11. (Ends were filled in with the Klenow fragment of DNA polymerase I or blunted with T4 DNA polymerase, as appropriate.) The *Eco*RI-*Sal*I fragment of pXY11 was then cloned into the cognate sites of pSH2-1. Codons 1 through 87 of *lexA* and seven codons originating from the polylinkers are fused in frame to codon 57 of *STH1*. The remainder of the *STH1* ORF and the translational stop codon are present.

pLexA-STH1(669-1330) contains the *Kpn*I-*Bam*HI fragment of pBL51 cloned into the *Eco*RI and *Bam*HI sites of pSH2-1. This results in the in-frame fusion of *lexA* codons 1 through 87 and four codons derived from the pSH2-1 polylinker to *STH1* codon 669. A stop codon from the vector lies 6 residues 3′ to codon 1330.

β-Galactosidase assays. Transformants were grown to mid-log phase in SC medium containing 2% glucose and lacking uracil and histidine to maintain selection for plasmids. β-Galactosidase activity was assayed in permeabilized cells (23) and is expressed as described by Miller (44).

Localization of STH1–β-galactosidase by immunofluorescence microscopy. pSTH1(1291)-lacZ contains the *Eco*RI-*Nco*I *STH1* fragment cloned in YEp353 (47). Diploid strain MCY2471 (*sth1-2::HIS3/STH1*) was transformed with pSTH1 (1291)-lacZ, and three Ura^+ transformants were sporulated with selection for the plasmid. The 16 tetrads examined yielded Ura^+ His^+ spores, indicating that pSTH1(1291)-lacZ complements *sth1*. Cells from a His^+ Ura^+ segregant (MCRY900) were prepared, stained, and photographed as described previously (64).

Immunoblot analysis. Immunoblot analysis of total cell proteins was carried out as described previously (8). LexA or β-galactosidase fusion proteins were detected by using rabbit polyclonal anti-LexA (a generous gift of Joanne Kamens and Roger Brent) or mouse monoclonal anti-β-galactosidase (Promega Biotec) antibodies, respectively. Primary antisera were detected by using either goat anti-rabbit or goat anti-mouse immunoglobulin G (Fc)- and heavy plus light chains-alkaline phosphatase conjugates and the Proto-Blot immunoblotting system (Promega Biotec).

Nucleotide sequence accession number. The nucleotide sequence accession number for the DNA sequence in this study is M83755.

RESULTS

Cloning a gene homologous to *SNF2*. To test for other sequences in the genome homologous to *SNF2*, we carried out Southern blot analysis of genomic DNA prepared from wild-type and *snf2Δ* strains. The filter was hybridized to a 1.1-kb fragment containing codons 835 to 1206 of the *SNF2* gene and washed under conditions of low stringency. In addition to the fragments derived from the *SNF2* locus, one or more strongly hybridizing bands and several fainter bands were detected in each lane (Fig. 1). The strongly hybridizing bands do not correspond to fragments predicted from the known region of homology adjacent to *LYS2* (*RAD16*) (38).

To clone homologous sequences, we used the 1.1-kb *SNF2* fragment to screen a yeast genomic DNA library (7). Approximately 7,500 bacterial colonies carrying plasmids were screened by colony hybridization, and 11 colonies hybridized strongly to the probe upon retesting. Of these, nine contain DNA from the *SNF2* locus, as judged by restriction site analysis. The remaining two clones are from another locus, and one completely encompasses the other. The smaller clone, pBL50 (Fig. 2), contains the entire coding

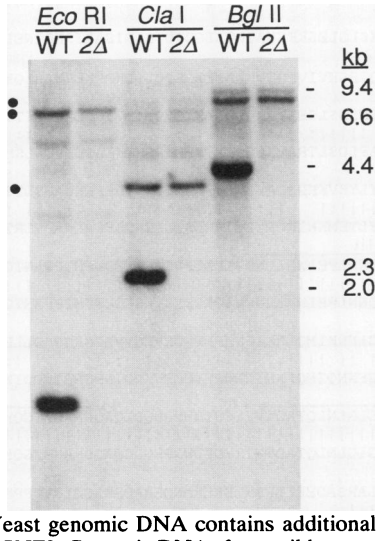

FIG. 1. Yeast genomic DNA contains additional sequences homologous to *SNF2*. Genomic DNAs from wild-type strain MCY1093 (WT) and *snf2*Δ mutant MCY1996 (2Δ) were digested with *Eco*RI, *Cla*I, or *Bgl*II, as indicated. DNAs were separated by electrophoresis on a 0.7% agarose gel, transferred to nitrocellulose paper, and hybridized to a ^{32}P-labeled *SNF2* fragment (see Materials and Methods). The strongly hybridizing 1.1-kb *Eco*RI, 2.2-kb *Cla*I, and 4.7-kb *Bgl*II fragments present only in the wild-type DNA are derived from the *SNF2* locus (3). ●, fragment present in both wild-type and *snf2*Δ DNA that most likely is derived from the *STH1* locus (Fig. 2). The 7.7-kb *Eco*RI fragment extends from the *STH1* site to the *KGD1* *Eco*RI site. *STH1* has an internal 3.7-kb *Cla*I fragment. The ~8-kb *Bgl*II fragment extends from the *STH1* site to a presumed *Bgl*II site 3′ to the *KGD1* gene. In addition, the 2.5-kb *Kpn*I fragment from the *STH1* locus was detected in both wild-type and *snf2*Δ DNA digests (data not shown). The sizes of marker fragments are shown.

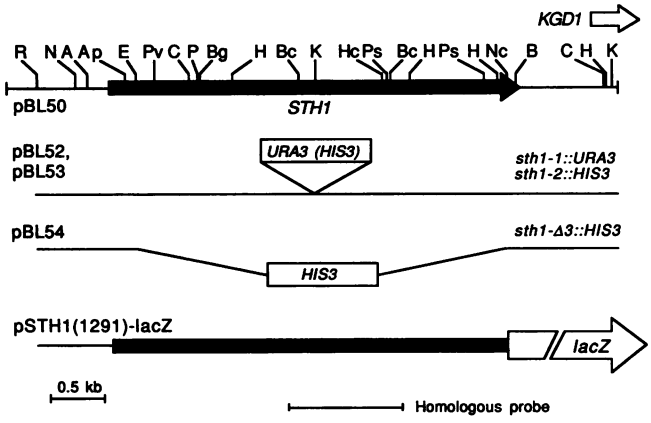

FIG. 2. Restriction maps of the *STH1* gene and plasmids. The direction of transcription is left to right. The placement of the *KGD1* gene is discussed in the text. Plasmids are described in the text. The nucleotide sequence for both strands was determined for the region extending from the *Eco*RI site to ~440 bp 3′ to the *Bam*HI site. The region of *STH1* homologous to the *SNF2* probe used in Fig. 1 is indicated. Restriction sites: A, *Acc*I; Ap, *Apa*LI; B, *Bam*HI; Bc, *Bcl*I; Bg, *Bgl*II; C, *Cla*I; E, *Eco*RV; H, *Hin*dIII; Hc, *Hin*cII; K, *Kpn*I; N, *Nhe*I; Nc, *Nco*I; P, *Pvu*II; Ps, *Pst*I; Pv, *Pvu*I; R, *Eco*RI.

region of a gene that we designate *STH1* for *SNF* two homolog. The restriction map of the cloned *STH1* locus is consistent with the sizes of the *Eco*RI, *Cla*I, *Bgl*II, and *Kpn*I fragments that hybridized most strongly to the *SNF2* probe on the genomic Southern blot (Fig. 1; also data not shown).

Sequence of *STH1* and homology of the predicted protein to SNF2. We determined the nucleotide sequence of 5.1 kb of the cloned *STH1* DNA, which includes an ORF of 1,352 codons (Fig. 3). The 369 nucleotides 3′ to this ORF are identical to the sequence 5′ to the *KGD1* gene (57) (Fig. 2). Analyses of the *STH1* RNA and *lexA-STH1* and *STH1-lacZ* fusion products (see below) confirm that this ORF corresponds to the *STH1* gene. The predicted protein has a molecular mass of 155,914 Da and comprises 33% charged residues, although the net charge is essentially neutral. The carboxyl terminus (residues 900 through 1352) is 41% charged and includes several short, predominantly acidic or basic regions.

The predicted STH1 protein is strikingly similar to SNF2 over a large region. Alignment of the deduced amino acid sequences of the STH1 and SNF2 proteins by using the BESTFIT program (67, 68) of the Genetics Computer Group (University of Wisconsin) (12) indicates that residues 351 through 987 of STH1 are 72% identical to residues 629 through 1289 of SNF2 (Fig. 3). In addition, residues 1270 through 1335 of STH1 are 46% identical to residues 1575 through 1640 of SNF2. STH1 also contains two repeated sequences, QLLEK at residues 123 through 127 and 311 through 315 and AGKFD/E at residues 790 through 794 and 942 through 946, which are both completely conserved in SNF2.

NTP binding and helicase motifs in STH1 and SNF2. The STH1 and SNF2 proteins contain sequences matching the A and B consensus sites of the bipartite NTP-binding motif (79). The sequence ILADEMGLGKTIQSI at residue 492 of STH1 corresponds to the A site, and the sequence IIDEGH RMK at residue 595 is homologous to the D-E-A-H/D box, a version of the B site that is characteristic of a superfamily of ATP-dependent helicases (underlined amino acids match consensus sequences) (22, 41, 79). The invariant K residue within the A box can interact with the γ phosphate of either ATP or GTP (13, 15, 55), and the conserved D residue of the B motif is thought to interact with the Mg^{2+} cation of Mg-NTP (13).

The STH1 and SNF2 proteins contain several additional sequences that are very similar to the conserved segments of two related superfamilies of proteins with determined or presumed helicase activities (21, 22, 31, 41). Besides motifs I and II, equivalent to the A and B boxes of the NTP-binding motif, STH1 and SNF2 have appropriately spaced sequences corresponding to motifs Ia, III, IV, V, and VI of the seven conserved motifs found in two superfamilies of more than 25 DNA and RNA helicases (Fig. 3) (22, 31, 82). The STH1 and SNF2 proteins are also homologous to other proteins from a wide range of eukaryotes and may define a new family of proteins related to helicases (see Discussion and Fig. 7 and 8).

Identification of the *STH1* RNA. The *STH1* RNA was identified by Northern (RNA) blot analysis. The internal 1.7-kb *Hin*dIII fragment of pBL50 (Fig. 2) was used to probe poly(A)-containing RNAs prepared from wild-type strains. Using stringent hybridization and washing conditions, we detected a 4.4-kb RNA, which is large enough to encompass the *STH1* ORF of 1,352 codons (Fig. 4, lanes a and b). This species was also present in RNA from *snf2*Δ mutants (lanes c and d).

***STH1* is essential for viability.** To determine the phenotype of an *sth1* null mutation, we introduced the insertion muta-

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STH1                              MLQEQSELMSTVMNNTPTTVAALAAVAAASETNGKLGSEEQPEITIPKPRSSAQLEQLLYRYRAIQNHPKENKLEIKAIEDTFRNISRDQDIY      93

STH1  ETKLDTLRKSIDKGFQYDEDLLNKHLVALQLLEKDTDVPDYFLDLPDTKNDNTTAIEVDYSEKKPIKISADFNAKAKSLGLESKFSNATKTALGDPDTEIRISARISNRINELERLPANL  213

STH1  GTYSLDDCLEFITKDDLSSRMDTFKIKALVELKSLKLLTKQKSIRQKLINNVASQAHHNIPYLRDSPFTAAAQRSVQIRSKVIVPQTVRLAEELERQQLLEKRKKERNLHLQKINSIIDF  333

STH1  IKERQSEQWSRQERCFQFGRLGASLHNQMEKDEQKRIERTAKQRLAALKSNDEEAYLKLLDQTKDTRITQLLRQTNSFLDSLSEAVRAQQNEAKIL......HGEEVQPIT........  438
                           ||     | ::|:||||| |: ||:|| ||| ||||||:||||||||||| |||||| |||||: ||: ||    | :          |||: ::
SNF2                       FGHRLIATHTNLERDEQKRAEKKAKERLQALKANDEEAYIKLLDQTKDTRITHLLRQTNAFLDSLTRAVKDQQKYTKEMIDSHIKEASEEVDDLSMVPKMKDE  731
                                                                     I                       Ia
STH1  ....DEEREKTDYYEVAHRIKEKIDKQPSILVGGTLKEYQLRGLEWMVSLYNNHLNGILADEMGLGKTIQSISLITYLYEVKKDIGPFLVIVPLSTITNWTLEFEKWAPSLTTIIYKGTP  554
          |::     |||:|||||||| | ||||||||||||:||::||:||||||:||||||||||||||||||||||:|||:||||||:|     ||:|||||||||::||: || ||||:| || :||:|
SNF2  EYDDDDDNSNVDYYNVAHRIKEDIKKQPSILVGGTLKDYQIKGLQWMVSLFNNHLNGILADEMGLGKTIQTISLLTYLYEMKNIRGPYLVIVPLSTLSNWSSEFAKWAPTLRTISFKGSP  851
                                       II                               III
STH1  NQRHSLQHQIRVGNFDVLLTTYEYIIKDKSLLSKHDWAHMIIDEGHRMKNAQSKLSFTISHYYRTRNRLILTGTPLQNNLPELWALLNFVLPKIPNSAKTFEDWFNTPFANTGTQEKLEL  674
      |:|    |  ||  |:|||:|||:||||||:: ||||  |  |||||||||||||||| |:     |     ||||||||||||||||||||||||||||| ||  |:|::||||||||||||  |:|:||
SNF2  NERKAKQAKIRAGEFDVVLTTFEYIIKERALLSKVKWVHMIIDEGHRMKNAQSKLSLTLNTHYHADYRLILTGTPLQNNLPELWALLNFVLPKIFNSVKSFDEWFNTPFANTGGQDKIEL  971
                        IV
STH1  TEEETLLIIRRLHKVLRPFLLRRLKKEVEKDLPDKVEKVIKCKLSGLQQQLYQQMLKHNALFVGAGTEGATKGGIKGLNNKIMQLRKICNHPFVFDEVEGVVNPSRGNSDLLFRVAGKFE  794
      :||||||:||||||||||||||||||||||:|||:||||||||:|||:|:||| :||||||      ||:|     :         |::|  ||  ||||:||||||||||:|||   :||:|    |  ::|||||||
SNF2  SEEETLLVIRRLHKVLRPFLLRRLKKDVEKELPDKVEKVVKCKMSALQQIMYQQMLKYRRLFIG.DQNNKKMVGLRGFNNQIMQLKKICNHPFVFEEVEDQINPTRETNDDIWRVAGKFE  1090
                                                                           V                                 VI
STH1  LLDRVLPKFKASGHRVLMFFQMTQVMDIMEDFLRMKDLKYMRLDGSTKTEERTEMLNAFNAPDSDYFCFLLSTRAGGLGLNLQTADTVIIFDTDWNPHQDLQAQDRAHRIGQKNEVRILR  914
      ||||:||| ||:|||||:||||||:||||||||    ::||:|||| ||::||:|:|  |||||:| ||:||||||||||||||||||||||||||||||||||||||||||||||||||||||||
SNF2  LLDRILPKLKATGHRVLIFFQMTQIMDIMEDFLRYINIKYLRLDGHTKSDERSELLRLFNAPDSEYLCFILSTRAGGLGLNLQTADTVIIFDTDWNPHQDLQAQDRAHRIGQKNEVRILR  1210

STH1  LITTDSVEEVILERAMQKLDIDGKVIQAGKFDNKSTAEEQEAFLRRLIESETNR......DDDDKAELDDDELNDTLARSADEKILFDKIDKERMNQERADRKAQGLRVPPPRLIQLDEL  1028
      ||||:||||||||||    ||||||||||||||||||| ||||| || |:: | :|.          ::  || | |:|: |||
SNF2  LITTNSVEEVILERAYKKLDIDGKVIQAGKFDNKSTSEEQEALLRSLLDAEEERRKKRESGVEEEEELKDSEINEILAR                                        1289

STH1  PKVFREDIEEHFKKEDSEPLGRIRQKKRVYYDDGLTEEQFLEAVEDAIKKRREARERRRLRQNGTKENEIETLENTPEASETSLIENNSFTAAVDEETNADKETTASRSKRRSSRKKRTI  1148

STH1  SIVTAEDKENTQEESTSQENGGAKVEEEVKSSSVEIINGSESKKKKPKLTVKIKLNKTTVLENNDGKRAEEKPESKSPAKKTAAKKTKTKSKSLGIFPTVEKLVEEMREQLDEVDSHPRT  1268

STH1  SIFEKLPSKRDYPDYFKVIEKPMAIDIILKNCKNGTYKTLEEVRQALQTMFENARFYNEEGSWVYVDADKLNEFTDEWFKEHSS                                     1352
       ||   |||  ||||: :|  |:| | |          | :| |  |      :| ||| || ||| || |
SNF2   IFLSKPSKALYPDYYMIIKYPVAFDNINTHIETLAYNSLKETLQDFHLIFSNARIYNTEGSVVYED                                                     1640
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FIG. 3. The predicted amino acid sequence of STH1 and alignment with homologous regions of the SNF2 protein. The amino acid sequences are given in standard single-letter code. The 5.1-kb nucleotide sequence has been deposited in GenBank. Numbering of the amino acid residues is on the right; proteins are identified on the left. Stop codons were found in all three frames 5′ to the presumed translational initiation codon. Subsequent construction of gene fusions to *lacZ* and *lexA* confirmed the reading frame. Regions of the SNF2 protein that are homologous to STH1 are shown, and alignment was by the BESTFIT program of the Genetics Computer Group package (12, 67, 68) with minor modifications. Vertical lines between the sequences indicate identity, and two dots indicate conservative amino acid changes (amino acids from the following groups were considered similar: L, V, I, and M; G and A; S and T; K and R; D, E, N, and Q; and F, Y, and W). Gaps in the STH1 and SNF2 sequences have been inserted to improve the alignment. Bold overlines indicate putative helicase motifs (see the legend to Fig. 8).

tion *sth1-1::URA3* (Fig. 2) on one chromosome homolog of the diploid strain MCY1751 (see Materials and Methods). Three Ura^+ disruptants were sporulated at 25°C. Sixteen tetrads were dissected, and the spores were germinated at 30°C on rich medium (YPD). Only two spore clones were viable from each ascus, and all viable clones were Ura^-. Similar viability patterns were observed when spore clones were germinated at 25°C. Inviable spores germinated and divided on the average 2 to 3 times, indicating that the *STH1* gene is essential for the mitotic growth of yeast cells.

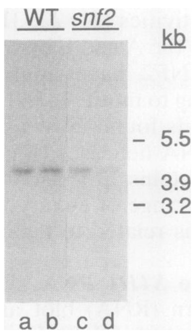
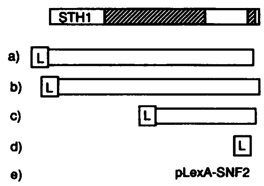


FIG. 4. Northern analysis of the *STH1* RNA. Poly(A)-containing RNA was prepared from wild type (WT) and *snf2* mutant strains. Lanes: a, MCY1094 (*MATα SNF2*); b, MCY1093 (*MAT***a** *SNF2*); c, MCY1998 (*MATα snf2Δ*); d, MCY1997 (*MAT***a** *snf2Δ*). RNAs were separated in a 1.5% agarose gel containing formaldehyde, transferred to nitrocellulose, and hybridized to the nick-translated 1.7-kb *Hin*dIII fragment of pBL50. The filter was also hybridized to a *TUB2* probe (49) as a control for the amount of RNA in each lane. Sizes of marker DNA fragments are indicated.



	Target Gene Activation		
	pLR1Δ1	1840	pSH18-18
a)	0.3	2.1	3.0
b)	0.1	0.3	0.3
c)	0.2	1.3	0.9
d)	ND	0.2	0.7
e) pLexA-SNF2	0.2	120	350

FIG. 5. Assay of LexA-STH1 fusion protein for activation function. The host strain was MCY829 (*his3 ura3*). A schematic representation of the STH1 protein is shown along with regions homologous to SNF2 (▨). L, LexA DNA-binding domain (residues 1 to 87) derived from vector pSH2-1 (not drawn to scale). Rows: a, pLexA-STH1(1-1330); b, pLexA-STH1(57-1352); c, pLexA-STH1(669-1330); d, vector pSH2-1; e, pLexA-SNF2 expressing residues 14 to 1696 of SNF2 (40). The target plasmids 1840 and pSH18-18 (6, 16, 26) are derived from pLR1Δ1 (83) and contain one and multiple *lexA* operators, respectively, inserted at position −167 relative to the *GAL1* transcriptional start site. The upstream activating sequence (UAS_G) is deleted. Transformants were grown in SC medium lacking histidine and uracil to select for the expression and target plasmids. Values are the average of β-galactosidase activities in at least three, usually four, transformants. ND, not determined.

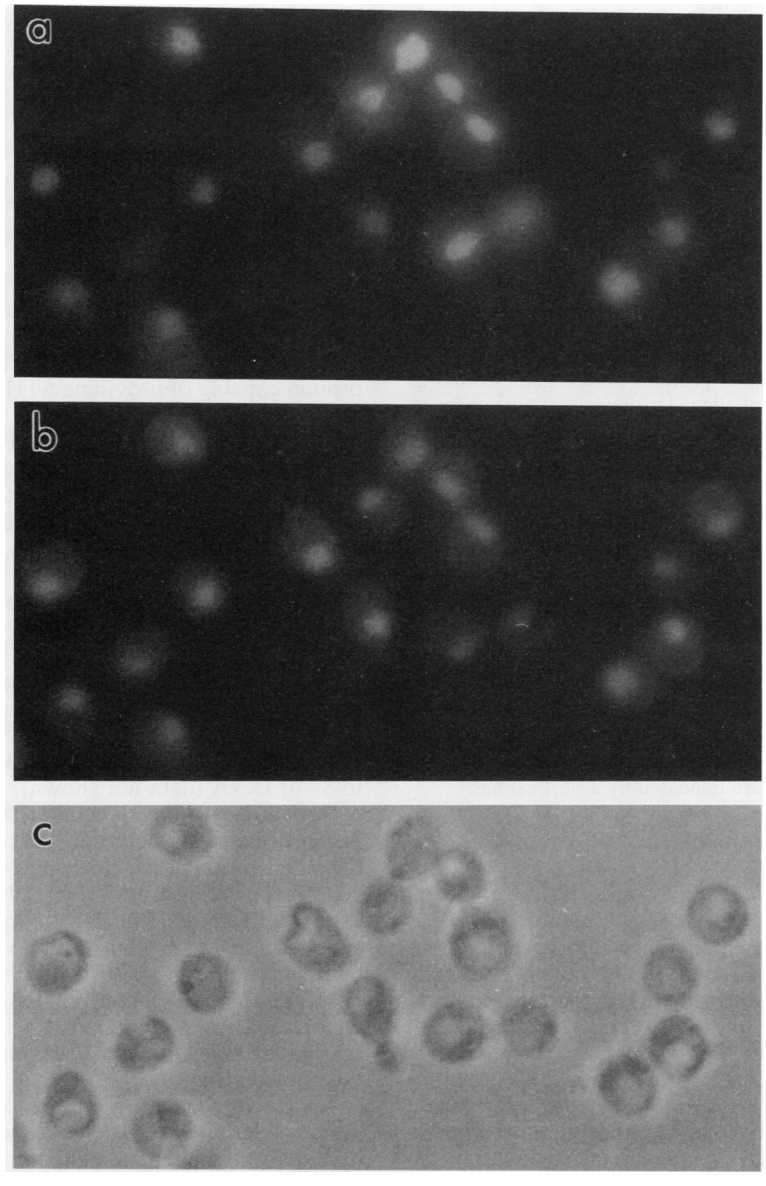


FIG. 6. Nuclear localization of the STH1–β-galactosidase fusion protein. Cells of strain MCRY900 carrying pSTH1(1291)-lacZ were grown to mid-log phase in SC-ura (2% glucose) medium and examined by immunofluorescence microscopy as described previously (64). (a) Cells stained with mouse monoclonal anti-β-galactosidase antibody (Promega Biotec) and the fluorescein isothiocyanate-conjugated $F(ab')_2$ fragment of sheep antibody to mouse immunoglobulin G (Sigma); (b) the same field of cells stained with 4',6-diamidino-2-phenylindole to identify nuclei and mitochondria; (c) phase-contrast field.

To confirm this result, the *sth1-1*::*URA3* and *sth1-2*::*HIS3* mutations were introduced into the diploid strain MCY2448. One His^+ transformant (MCY2471) and three Ura^+ transformants were sporulated at 30°C. Analysis of 5 and 16 tetrads, respectively, yielded in each case only two viable spores, which were His^- or Ura^-. To test whether *STH1* is essential in a genetic background different from that of S288C, the substitution mutation *sth1-Δ3*::*HIS3* was constructed in strain MCY2447, which is derived from W303. Each of four tetrads yielded two viable His^- spore clones. The *STH1* gene is therefore essential for viability.

***STH1* and *SNF2* are functionally distinct.** The sequence similarity between SNF2 and STH1 suggests that these proteins are functionally related. However, mutants lacking *SNF2* are unhealthy but not inviable. A possible explanation for the different mutant phenotypes is that the SNF2 and STH1 proteins are functionally redundant but expressed at different levels, with STH1 being more abundant. We therefore tested whether an increased dosage of *SNF2* or *STH1* could compensate for loss of the other gene. We first tested the ability of *SNF2* carried on a multicopy plasmid to suppress the lethality of the *sth1-2*::*HIS3* mutation. Plasmid pLN138-4 (3) was used to transform diploid strain MCY2471 (*sth1/STH1 ura3/ura3*), and two Ura^+ transformants were sporulated under conditions selective for the plasmid and subjected to tetrad analysis. All 14 dissected asci yielded only two viable spore clones, all of which carried the plasmid *URA3* marker, indicating that an in-

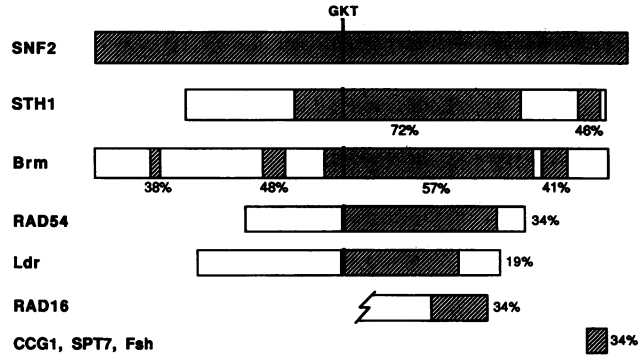


FIG. 7. Schematic alignment of proteins homologous to SNF2. The SNF2 protein and regions of aligned proteins that are similar to SNF2 are indicated (▨). The percentage of amino acid identity between each protein and SNF2, as determined by the BESTFIT program (67, 68), is indicated. Broken edges indicate that the sequence is incomplete. Homology is shown for the following regions: STH1 residues 351 to 987 and 1270 to 1335 to SNF2 residues 629 to 1289 and 1575 to 1640, respectively (40); Brm residues 750 to 1380 and 1441 to 1522 (78) to SNF2 residues 717 to 1396 (the same region is 59% identical to STH1 residues 447 to 1074) and 1566 to 1647, respectively; *lodestar* (Ldr) residues 461 to 840 (20) to SNF2 residues 788 to 1100; RAD54 residues 327 to 831 (17) to SNF2 residues 784 to 1263; the carboxy-terminal 170 residues of RAD16 (63) to SNF2 residues 1088 to 1263; the repeated Fsh residues 55 to 118 and 499 to 562 (28), CCG1 residues 1345 to 1406 and 1468 to 1531 (65), and SPT7 residues 2 to 57 (14) to SNF2 residues 1576 to 1631. The positions of the GKT residues of the A box of the NTP-binding motif are indicated by a vertical bar.

creased *SNF2* gene dosage does not compensate for loss of *STH1* function.

We next tested whether an increased dosage of *STH1* restores growth of an *snf2*Δ mutant on raffinose. Strain MCY1250 (*snf2*Δ) was transformed with the multicopy plasmid pBL50, and Ura$^+$ transformants were tested for anaerobic growth on SC medium containing 2% raffinose and lacking uracil. No differences in growth between transformants carrying pBL50 and those carrying the vector were detected. Together, these data suggest that the functions of *SNF2* and *STH1* are distinct.

Lethality of *sth1* is not suppressed by *spt6*. Mutations in the *SPT6/SSN20* gene suppress various defects caused by *snf2* mutations (27, 50–52). We therefore tested whether *spt6* suppresses the lethality caused by *sth1*. The *sth1-1*::*URA3* allele was introduced into the diploid MCY815 × MCY2484 (*ssn20-6/ssn20-1 ura3/ura3*) by transformation with a fragment from pBL52, as before. Tetrad analysis of two Ura$^+$ transformants yielded only two viable spores from each of nine tetrads. Thus, the *spt6/ssn20* mutation does not restore viability to *sth1* segregants. This evidence further supports the idea that the *SNF2* and *STH1* products have different roles in the cell.

DNA-bound LexA-STH1 fusion protein does not activate gene expression. Previous evidence strongly implicates SNF2 in transcriptional activation, and a LexA-SNF2 fusion protein tethered to DNA via a *lexA* operator activates expression of an adjacent *GAL1-lacZ* target gene (40). To test whether the STH1 protein functions similarly, we constructed three *lexA-STH1* fusions expressed from the *ADH1* promoter in the vector pSH2-1 (25) (Fig. 5). pLexA-STH1(1-1330) expresses the DNA-binding domain of LexA (residues 1 to 87) fused to residues 1 to 1330 of STH1. This bifunctional fusion protein migrates with the expected size on an SDS-polyacrylamide gel, as detected by immunoblotting, and provides *STH1* function in an *sth1* null mutant (see Materials and Methods). pLexA-STH1(57-1352) and pLexA-STH1(669-1330) express similar fusion proteins containing the indicated STH1 residues.

A wild-type strain was cotransformed with each of the pLexA-STH1 expression plasmids and target plasmid 1840 or pSH18-18 (6, 16, 26), which carry one and multiple *lexA* operators, respectively, located 5′ to the *GAL1-lacZ* promoter. None of the hybrid proteins significantly stimulated expression of β-galactosidase from the target gene (Fig. 5). In contrast, a LexA-SNF2 fusion protein activates target gene expression dramatically. Thus, a LexA-STH1 fusion protein capable of providing STH1 function in vivo does not function in this transcriptional activation assay in a manner comparable to that of LexA-SNF2.

DNA-bound LexA-STH1 does not repress activation of a target gene. We next considered the notion that STH1 might function antagonistically to SNF2. We tested the possibility that LexA-STH1 might repress transcription from target plasmid CK26 (34) or JK126 (33), which contain one and four *lexA* operators, respectively, inserted upstream of the *CYC1* UASs in pLGΔ312S (24) at position −324 with respect to the transcriptional start site. We cotransformed wild-type cells with pLexA-STH1(1-1330) and each of the target plasmids. Expression of the *CYC1-lacZ* target gene was not significantly different in transformants carrying pLexA-STH1(1-1330) or the vector pSH2-1, which expresses only residues 1 to 87 of LexA (data not shown).

Nuclear localization of a bifunctional STH1–β-galactosidase fusion protein. To determine the subcellular localization of the *STH1* product, we constructed a bifunctional *STH1-lacZ* gene fusion. Plasmid pSTH1(1291)-lacZ contains the *STH1* gene fused at codon 1291 to the *lacZ* gene (Fig. 2) and encodes a fusion protein of the expected size, as determined by immunoblotting. pSTH1(1291)-lacZ provides *STH1* function (see Materials and Methods). Immunofluorescent staining of *sth1-2*::*HIS3* mutant cells carrying the plasmid showed that the STH1–β-galactosidase fusion protein was located in the nucleus (Fig. 6).

A sequence matching the consensus for nuclear localization signals (NLS), AKKTK at residue 1232, was identified by using the Genetics Computer Group program MOTIFS (5). Near this sequence are potential phosphorylation sites for the histone H1 kinase activity of cdc2/CDC28 and for casein kinase II (CKII). The nuclear entry of the SWI5 transcription factor is regulated by the cell cycle-dependent CDC28 phosphorylation of sites in or near the SWI5 NLS (46). Putative CKII phosphorylation sites have been found near the NLS of many nuclear proteins, and it has been suggested that phosphorylation by CKII modulates the rate of nuclear transport (58) or regulates transport (20).

DISCUSSION

We have cloned and characterized *STH1*, an essential yeast gene, by virtue of its homology to *SNF2*. The predicted 156-kDa STH1 protein is strikingly similar to the SNF2 protein. The two are 72% identical over a region of 661 amino acids and 46% identical over another stretch of 66 amino acids. Despite this high degree of identity and the coincidence of the *STH1* and *SNF2* products in the nucleus, the two proteins appear by several criteria to be functionally distinct. First, increased dosage of *STH1* or *SNF2* does not compensate for loss of the other. Second, the mutation *spt6*,

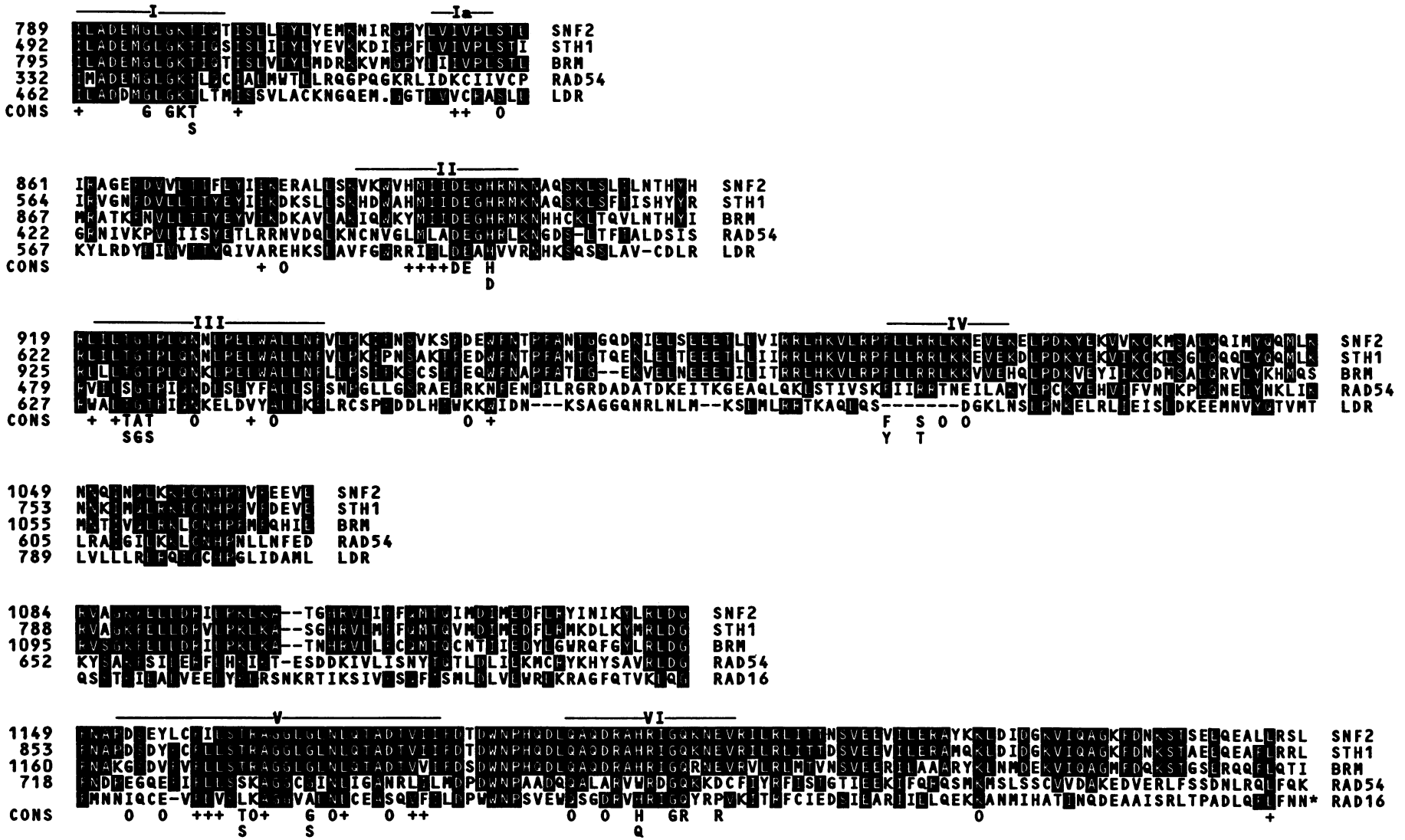


FIG. 8. Alignment of conserved regions of the SNF2, STH1, Brm, RAD16, RAD54, and Ldr proteins. The alignment of amino acid sequences from six regions of these proteins is shown. Sequences that correspond to the two NTP-binding motifs (79) and those that correspond to the five other consensus motifs identified in two superfamilies that include DNA and RNA helicases (21, 22, 31, 41) are overlined by heavy bars. These motifs are numbered by convention: I (corresponding to the A box of the NTP-binding motif), Ia, II (corresponding to the B box or the version referred to as the DEAD/H box), III, IV, V, and VI. The consensus (CONS) sequences are taken from Gorbalenya et al. (22), and hydrophobic residues (I, L, V, M, F, Y, and W) and charged or polar residues (S, T, D, E, N, Q, K, and R) are indicated (+ and O, respectively). The STH1 sequence that corresponds to segment IV is identifiable but degenerate. Residues boxed in black are identical in at least three of the sequences compared. References for the aligned sequences are as in the legend to Fig. 7. Alignments (and inserted gaps) were by the BESTFIT program (67, 68). The carboxyl terminus of RAD16 (*) is indicated. Ldr residues 489 to 524 (·) were deleted to improve the alignment.

which suppresses *snf2* defects, does not suppress the lethality caused by *sth1* mutations. Finally, the DNA-bound LexA-STH1 fusion protein, unlike LexA-SNF2, does not activate expression of a target gene.

The STH1 and SNF2 proteins share sequences matching the A and B boxes of the NTP-binding motif and can be considered putative NTP-binding proteins. Both proteins also contain sequences resembling the other five motifs that are conserved in two superfamilies of determined or presumed helicases (Fig. 3). These motifs are appropriately spaced in STH1 and SNF2, with the possible exception of motifs IV and V, which are approximately 150, rather than 50, residues apart.

The large region of homology shared by STH1 and SNF2, which includes the helicase motifs, is also conserved among several other eukaryotic proteins (Fig. 7). Most striking is the comparison to the *Drosophila melanogaster* Brm protein (78) (Fig. 7). Over a 630-residue region, Brm shows 57% identity to SNF2. The SNF2 and STH1 proteins are also homologous to the *S. cerevisiae* RAD54, RAD16, and MOT1 proteins (10, 17, 63) and the *D. melanogaster lodestar* product (20). Other partially characterized *Bombyx mori*, mouse, and human proteins share 27 to 58% identity to SNF2 in regions located between residues 605 and 1413 (11, 54, 70). In addition, outside the region of similarity to helicases, SNF2, STH1, and Brm share a small patch of homology with the *S. cerevisiae SPT7*, human *CCG1*, and *D. melanogaster fsh* gene products (14, 28, 65). This homologous sequence is repeated in both Fsh and CCG1, but an *SNF2-lacZ* fusion lacking this region is functional in multicopy.

The STH1 and SNF2 proteins appear to define a new family of proteins that are related to helicases. Within the large blocks of homology shown in Fig. 7, the regions of the greatest sequence conservation encompass the helicase motifs (Fig. 8). These conserved regions extend to either side of the motifs and differentiate this family from the previously described families of helicases. Moreover, the conservation of sequences surrounding the motifs lends support to the idea that these elements are functionally significant.

While the proteins homologous to SNF2 appear to constitute a new family, their identification as putative NTP-binding proteins or helicases rests solely on the presence of the signature motifs. No nucleotide binding or hydrolysis or helicase activity has been demonstrated for any of these proteins. However, the available evidence regarding the functional roles of these proteins is consistent with possible DNA-unwinding activity. The two described superfamilies of putative helicases include proteins involved in DNA and RNA replication, recombination, repair, RNA splicing, and translational initiation (21, 22, 31, 41, 79, 80). Moreover, the *D. melanogaster* Mle protein, which contains helicase motifs, is involved in transcriptional regulation (37), and the human RAP30/74 transcription initiation factor (TFIIF) is associated with an ATP-dependent DNA helicase activity (19, 69). Several of the proteins in the SNF2 family have functions related to transcription and repair.

The SNF2 protein has been implicated in transcriptional activation (40), and genetic evidence suggests that interactions of SNF2 with chromatin may be involved. Various *snf2/swi2* mutant defects are suppressed by a reduced dosage of the histone H2A and H2B genes (30); by mutations in *SPT5* and *SPT6*, which are thought to affect chromatin (76, 77); and by mutations in *SIN1*(*SPT2*), which encodes a protein that is similar to the mammalian HMG1 chromatin-associated protein (36) and that may interact with RNA polymerase II (56). These data can be reconciled with helicase activity for SNF2.

Tamkun et al. suggest a role for Brm in transcriptional activation (78). Mutations in *brahma* (*brm*) are dominant suppressors of mutations in *Polycomb*, a regulator of homeotic genes in *D. melanogaster* (35). The *brm* gene is required during development for activation of multiple homeotic genes (78).

RAD54 and *RAD16* are involved in the recombinational and excision repair of DNA damage, respectively (17, 63). DNA repair in prokaryotes and eukaryotes requires helicase activity (43, 74). The *S. cerevisiae RAD3* gene, which is required for excision repair of DNA, encodes a DNA-dependent ATPase with DNA and DNA-RNA helicase activities (4, 74, 75), and other eukaryotic genes with roles in DNA repair encode known or putative helicases (HPR5/RADH, ERCC-2, ERCC-3) (1, 59, 81, 82). Thus, the possibility that *RAD16* and *RAD54* encode helicase activities is compatible with their known functional roles in repair.

Mutations in *lodestar*, a maternal-effect gene, cause chromatin bridges at anaphase, which have been suggested to result from defects in the mechanism for moving chromatids to opposite poles at anaphase, incomplete DNA replication or repair, or abnormalities in chromatin condensation (20). Although an NTP-hydrolyzing or DNA-unwinding activity could be involved in one of these processes, the *lodestar* gene product does not include the recognizable helicase motifs IV, V, and VI (20).

We have as yet no insight into the functional role of STH1. Unlike SNF2, STH1 did not affect transcription in the assays we used. Because *STH1* is an essential gene, conditional-lethal mutations will be required to identify its function.

Thus, SNF2 and STH1 appear to define a new family of proteins that are related to helicases. The family includes proteins from a wide range of eukaryotes. Some of the proteins in this family function in processes that could require helicase activity, but their definitive identification as helicases will require further biochemical studies.

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